KARCH & ASSOCIATES, INC.

May 16, 1998

BY FEDERAL EXPRESS
Dr. C.W. Jameson
National Toxicology Program
Report on Carcinogens
MD EC-14
P.O. Box 12233
Research Triangle Park, NC 27709

Dear Bill:

Enclosed are comments on the recommended listing of trichloroethylene prepared by Karch & Associates, Inc., and Terra, Inc. We address scientific issues in the proposed listing of trichloroethylene, and we make a number of recommendations to you in the final determination of whether and how to list trichloroethylene. In addition to myself, the following individuals were involved in preparing these comments: Karyn Hentz, Senior Staff Scientist, and Darryl P. Arfsten, Staff Scientist, both at Karch & Associates and Rob James, President, Terra, Inc. We hope that our comments are helpful to you in your work on the Ninth *Report on Carcinogens*.

We would be pleased to answer any questions about our comments that you may have or about the literature cited. I can be reached at (202) 463-0400 by telephone or (202) 463-0502 by facsimile. If you would find it more convenient, I can be contacted by E-mail at the following address:

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We hope that our comments are helpful.

Sincerely,

Nate Karch

Nathan J. Karch, Ph.D., D.A.B.T.
President

Enclosure

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COMMENTS IN RESPONSE TO NTP CLASSIFICATION OF TRICHLOROETHYLENE AS *"Reasonably anticipated to be a human carcinogen"*

I. EXECUTIVE SUMMARY

The Review committees and the NTP Outside Peer Review Subcommittee have proposed classification of TCE as *reasonably anticipated to be a human carcinogen.* The basis for such classification by NTP is extremely limited data and relies inappropriately on structural analogs. Review of structural analogs can be helpful in some circumstances. However, when the data for a chemical are extensive, reliance on structural analogs is unnecessary and in many cases erroneous. More importantly, when differing mechanisms of tumor induction exist among the analogs there is strong reason to avoid this approach when assessing potential carcinogenicity. The data on carcinogenicity and mechanism for TCE are extensive and the mechanism of tumor induction differs among the compounds that NTP relied on as structural analogs of TCE. Thus, NTP's reliance on structural analogs is inappropriate.

NTP appears to rely heavily on certain kidney cancer literature, and IARC's conclusion that exposure to TCE is associated with elevated risks for liver and biliary tract cancer and non-Hodgkin's lymphoma. Some of the limitations and uncertainties associated with these data are covered in the background report, but we do not believe they are adequately described or evaluated. NTP has focused their attention on the positive findings of both epidemiological and experimental animal data. While this is useful in summarizing the data for a particular compound, it does not provide an adequate basis for a weight of the evidence assessment. Consequently, NTP has failed to assess properly the full range of available data on TCE, often placing too much emphasis on findings that support an association while ignoring others that do not. A weight of the evidence approach is necessary to evaluate the data for TCE in an unbiased fashion.

Based on our review, we believe that there are data to suggest that some of the cancers in animals, particularly the kidney and liver cancers, are a threshold, high-dose phenomenon to the extent that they are truly associated with TCE, if at all. The human data reflecting an increased incidence for both of these cancers are found in workers with the highest levels of exposure. In addition, animal data suggest that mechanisms for the induction of these cancers occur when the predominate metabolic pathways are overwhelmed in circumstances of high exposures. Overall, there are questions regarding the relevance of high dose findings in the occupational studies to low level exposures, such as drinking water contamination.

NTP also relies on the experimental animal data as predating and supporting the human data (p. RC-1). The literature cited refers to the observation of liver, lung, and lymphoma tumors in mice, and kidney and testicular tumors, and possibly leukemias in rats. In the text of the report,

there are discussions of the limited relevance to humans of liver and lung tumors in mice and kidney tumors in rats. These qualifications, however, are not adequately discussed, nor are they included in the Executive Summary of the classification of TCE.

In conclusion, we propose that any discussion and reliance on structural analogs be removed from the assessment of the potential carcinogenicity of TCE. We also propose that the limitations of the underlying data on TCE be described in greater detail in the Executive Summary in order to provide a more balanced presentation of the available data. Specifically, we believe that the following issues should be addressed. The findings for liver and biliary cancer, non-Hodgkin's lymphoma, and kidney cancer in the occupational cohorts are inconsistent. Bias, chance, and confounding have not been excluded as explanations for the small, observed elevations. Other factors, such as the lack of dose-response and small numbers of cancers limit the possible interpretations of these data. There are also numerous questions about the relevance of the animal tumors to humans, chief among them are differences observed among various species and genders and the mechanistic data that suggests that these differences are restricted to high dose or attributable to metabolic pathways not relevant to humans. In addition, there are limitations in the animal data because of the methodologies employed and the rate of survival in the experimental animals. Finally, if NTP still concludes that there exists any association between TCE exposure and the induction of cancer, the data supporting the proposition that TCE-induced carcinogenicity is a threshold, high dose phenomenon should be mentioned specifically.

II. STRUCTURAL ANALOGS

In addition to the data presented on TCE, NTP presents substantial information on three other compounds, vinyl chloride, 1,1-dichloroethylene (vinylidene chloride), and perchloroethylene as structural analogs of TCE (pp. 6-8). NTP's listing criteria from the Draft Background Report for TCE (p. LC-1), indicates that chemical structure is a point of consideration for determining carcinogenicity, noting that where there is less than sufficient evidence of carcinogenicity, structural analogs may be used for determining carcinogenicity. While these compounds may be structurally similar, there is no basis to support a mechanistic similarity among these compounds. In fact, we present data below that demonstrates that the mechanistic action of these compound differ substantially and the data for one compound should not be used to assess the effects of another. In a review of the carcinogenic potential of TCE and other anesthetics, Baden and Rice (1994) indicate that while structural similarities may be interesting, they do not provide proof of carcinogenic potential. The authors state that TCE is similar in structure to "the human and animal carcinogen" vinyl chloride but note:

> "Although these observations on structure are interesting, they are by no means proof that anesthetics have carcinogenic potential. Minor structural differences often impart major changes in function, as has been clearly shown with aromatic hydrocarbons. Epidemiological surveys, animal studies, and in vitro carcinogenicity assays provide more definitive evidence of carcinogenic potential." (Baden and Rice 1994, pp. 174-175.)

In addition to the general issue of use of structural analogs, there is considerable data on TCE itself that extrapolation of carcinogenicity from other compounds is unnecessary and only complicates the assessment of TCE. Therefore, the data on the structural analogs should not be used to evaluate the potential carcinogenicity of TCE.

Vinyl Chloride

Vinyl chloride may be structurally similar to TCE, but it is inappropriate to analyze the potential carcinogenicity of TCE based on such similarities. First, the signature carcinogenic lesion from vinyl chloride exposure is angiosarcoma of the liver, which is a unique type of liver cancer that has only been associated with exposure to vinyl chloride and none of the other ethylenes. Vinyl chloride is also recognized as a direct acting mutagen and TCE is, at most, weakly mutagenic. Thus, there is a lack of mechanistic data to support the use of vinyl chloride as a surrogate for TCE.

In a review of the chloroethylenes, Green (1990) explains that the discovery that vinyl chloride was carcinogenic in the early 1970's led to the assumption that the other chloroethylenes would be similarly carcinogenic on the basis of similarities in chemical structure. However, while Green (1990) notes that the analogy to vinyl chloride initially appeared appropriate because each chloroethylene was carcinogenic in at least one animal species, he states :

> "At this point, the similarities between vinyl chloride and the other chloroethylenes end. The expectation that they act in the same way as vinyl chloride through mutagenic epoxide metabolites has not been fulfilled. New mechanisms have been discovered involving peroxisomes, hyaline droplets, and new metabolic pathways that were unknown in the 1970s when vinyl chloride was first investigated. Many of the new mechanisms are believed to be nongenotoxic: they exhibit thresholds and in many cases show marked species-specificity." (Green 1990, p. 84.)

Vinyl chloride exposure produces angiosarcoma of the liver in occupationally exposed humans and has been induced in rats, mice, and hamsters (IARC 1979). Angiosarcoma of the liver is a rare type of liver tumor that has been associated with exposure to vinyl chloride, thorotrast, and arsenicals (in pesticides). In fact, the registry of angiosarcomas has not reported a single new case since 1988. This dramatic reduction in angiosarcomas has been attributed to a corresponding reduction in occupational exposure to vinyl chloride with the use of closed systems. The occurrence of angiosarcoma of the liver in vinyl chloride-treated animals is the result of DNA alkylation, which is a genotoxic event. In contrast, the only liver tumors that have been induced in TCE-exposed animals are hepatocellular carcinomas in B6C3F1 mice. Not only are these a different tumor type, but there is some question about the relevance of these tumors to humans because of the high background rate of liver cancer in this strain of mouse and the role peroxisome proliferation plays in the induction of these tumors.

Vinyl chloride is a direct mutagen and is considered to be a "complete" carcinogen. In other words, vinyl chloride is considered to be both an initiator and a promoter in the carcinogenic process. TCE can be considered, at most, weakly mutagenic. For example, IARC concluded that:

> "[P]ure trichloroethylene did not induce gene mutation in human cells. In mammalian cells *in vitro*, pure trichloroethylene induced cell transformation, sister chromatid exchange and gene mutation, but not chromosome aberrations." (IARC 1995a, p. 136)

Other reviewers have come to similar conclusions:

> "In conclusion, pure TCE is non-mutagenic in the majority of *in vitro* mutagenicity systems ... the weakly positive findings were observed only at high dose levels with no evidence of reproducibility in or between laboratories. (ECETOC 1994, p. 36)

> "Since TCE seems to have very specific genotoxic effects, it is apparent that it is not a typical genotoxic carcinogen (showing activity in a battery of standard genotoxicity tests). *In vitro* and *in vivo*, TCE induces recombination (including sister chromatid exchanges) and aneuploidies (including micronuclei), but it

seems un[cap]able of inducing gene mutations and structural chromosomal aberrations." (Fahrig et al. 1995, p. 32)

1,1-Dichloroethylene (Vinylidene Chloride)

NTP includes 1,1-dichloroethylene or vinylidene chloride (DCE) as a structural analog for TCE. It is not clear why NTP would refer to data on DCE when assessing the potential carcinogenicity of TCE because IARC (1987) concluded that DCE is not classifiable as a human carcinogen. There has been one epidemiology study conducted on DCE. Ott et al. (1976) found that there was no increased risk of cancer among 138 workers exposed to DCE. However, IRIS notes that this study is inadequate for assessing cancer risk in humans based on limited statistical power. Eighteen chronic animal bioassays have been conducted for vinylidene chloride and only one reported a carcinogenic response. Maltoni et al. (1985) reported a statistically significant increase in kidney adenocarcinoma in male mice. In addition, there is no basis for concluding that DCE is mutagenic or clastogenic in rodent species *in vivo* or in Chinese hamster ovary cells *in vitro* (IARC 1987). Therefore, the literature available on DCE-induced carcinogenicity does not support the hypothesis that TCE is carcinogenic in humans.

Perchloroethylene

Perchloroethylene (PCE) was also considered a structural analog by NTP, but is mechanistically distinct from TCE. The mechanism of action for PCE-induced kidney tumors and the mutational frequencies induced by PCE within the H- and K-*ras* genes demonstrate that small structural differences can have large impacts on the metabolism and mechanism of action for a compound. Therefore, PCE as a structural analog is not useful in assessing the potential carcinogenicity of TCE.

Small, but significant increases in renal cell tumors, have been observed in TCE cancer bioassays (Maltoni et al. 1988; NTP 1988, 1990) and low incidences of renal tubular cell cancer have been reported in PCE cancer bioassays (NCI 1977, NTP 1986). PCE induces the formation of kidney tumors in male rats through a non-genotoxic mechanism, via α-2μ-globulin formation in the kidney (Goldsworthy et al. 1988). However, when rats were treated with TCE, kidney α-2μ-globulin levels were similar to controls suggesting that rats do not form kidney tumors by this pathway (Goldsworthy et al. 1988). PCE clearly differs from TCE in the carcinogenic mechanism of action for kidney tumors demonstrating that these chemicals are not analogous in terms of their carcinogenicity.

Anna et al. (1994) found that PCE induces different mutation frequencies within the H- and K-*ras* genes of hepatocellular tumors compared with TCE or dichloroacetic acid. They state that "the mode of tumor induction may differ for tetrachloroethylene." The transversions in the H-*ras* gene following TCE exposure are similar to those produced in spontaneous liver tumors. PCE also induced a large number of K-*ras* mutations that "may be a chemically related effect." This

difference in mutation spectra is strongly indicative of mechanistic differences between TCE and PCE.

The mechanistic differences in the formation of male rat kidney tumors and the difference in mutation frequencies in hepatocellular carcinomas between PCE and TCE illustrate the dramatic impact a single chlorine atom can have on the mechanism of a compound. Given the scope of these differences, the use of PCE as a structural analog for TCE is inappropriate.

Limitations of Structural Analogies

As stated earlier, the primary use of structural analogies or structure activity relationships is in those situations where limited toxicity data are available for a particular compound and the toxicological endpoint of interest has not been evaluated. Again, this is not the case for trichloroethylene. Perhaps a better illustration of the potential futility of relying upon structural similarities as the basis for predicting toxic outcomes is to consider some examples of structure activity relationships reported for chemicals whose structures are even closer than the chlorinated alkene series considered by NTP.

For example, 1- and 2-nitropropane are two chemicals whose chemical molecular composition is identical, the only difference between these two compounds being the position of the nitro group on the propane chain. Thus, these two chemicals are of closer structural similarity than that of any of the four chemicals NTP considered in the TCE, DCE, PCE and vinyl chloride series. In addition, both 1- and 2-nitropropane are genotoxic, something the chlorinated alkene series does not have in common, and a feature which normally would suggest both are likely to be carcinogenic. Normally, if one were to predict *a priori* if two such structurally similar genotoxic compounds would be animal carcinogens if one of the isomers was found to be carcinogenic in animals, it is probably fair to say most scientists would agree a prediction of similar toxicities (carcinogenicity) based on known structure and activity considerations would have a high degree of probability. At least, the greater similarity of 1- and 2-nitropropane would have a better predictability than when comparing chemicals of different chemical compositions and different genotoxic activity (e.g., comparing vinyl chloride to TCE). However, as Cunningham et. al. (1991) have shown, only the hepatotoxic congener of the two, 2-nitropropane, was a liver carcinogen in rodents. The carcinogenic response of 2-nitropropane correlated with a cytotoxicity induced hepatocellular proliferation, an effect that the noncarcinogen, 1-nitropropane, did not induce even though it is genotoxic.

A similar paradox is observed when the structurally related and equally genotoxic compounds 2,4- and 2,6-diaminotoluene are evaluated for carcinogenicity. The 2,4-diaminotoluene isomer was the only one of the two isomers proved to be a hepatocarcinogen in both mice and rats, the 2,6-diaminotoluene was not carcinogenic in either species. Cunningham et al. (1991) have also demonstrated that while both series of compounds were genotoxic, carcinogenic activity correlated better with cytotoxicity.

Thus, with these two examples neither the predicted outcome or responsible mechanism are easily or accurately predicted on the basis of structural similarities, and again these two series of compounds are structurally more similar than TCE is to PCE, DCE or vinyl chloride. In short, structure activity relationships are useful to making screening judgements as to what type of toxicity studies might be considered when data is limited. However, structure activity predictions are predictions of highly variable and unknown reliability, and have no place in a chemical's evaluation when considerable human, animal, and mechanistic studies exist for a particular endpoint as is the case with TCE.

Summary

We do not believe that a structural analogy to vinyl chloride, perchloroethylene, and 1,1-dichloroethylene is scientifically supportable. It is unnecessary or inappropriate in the assessment of the carcinogenic potential of TCE. The three compounds have similarities in structure and even to a degree in the affected sites, but differ significantly from a mechanistic perspective. The mere fact that the carcinogenicity for these three compounds ranges between vinyl chloride as a known human carcinogen to 1,1,-dichloroethylene as unclassifiable, does not support the inclusion of these data in the assessment of TCE. In addition, and more importantly, the mechanistic data for vinyl chloride, perchloroethylene, and possibly 1,1-dichloroethylene as well, does not support a common mechanism for carcinogenicity. Since, the discussion of structural analogs does not contribute to the assessment of the potential carcinogenicity of TCE, we recommend that it be removed from the final NTP assessment of TCE.

III. LIMITATIONS OF THE DATA USED IN THE ASSESSMENT OF TCE

NTP cites several cancer sites as the basis for their classification of TCE as "reasonably anticipated to be a human carcinogen." There is a dearth of discussion regarding the limitations and inconsistencies in both the epidemiological and experimental animal data. We have identified several issues for each cancer type that should be included in the detailed discussion on TCE and highlighted in the Executive Summary.

A. LIVER CANCER

In its overall evaluation for TCE, liver cancer is one of the two cancers that IARC noted based upon human data and the primary site based on animal data. IARC considered the data on liver and biliary cancer in humans to show elevated risks. IARC characterized the human data as *limited evidence*. The mouse liver data that were cited for support of biological plausibility of an association between TCE exposure are of questionable relevance to humans because of the possible role of peroxisome proliferation in the induction of mouse liver tumors. Other mechanisms that reinforce the species-specificity of the induction of liver tumors to mice were not fully reviewed by IARC. However, the induction of tumors at other sites in experimental animals led IARC to conclude that the data in animals constituted *sufficient evidence* (see sections on kidney and lung tumors).

IARC classified TCE as a 2A carcinogen, and some of the limitations in the data were delineated. NTP relies heavily on the assessment by IARC, but provides only a limited discussion of the data linking TCE exposure and liver cancer. We believe that NTP failed to address adequately the limitations in the human data and address adequately the mechanistic data on TCE and its metabolites that strongly support species specificity in the induction of mouse liver tumors.

Human Data

The NTP employs different classification categories than IARC. This difference leads to problems that arise chiefly because NTP's evaluation of TCE exposure and liver and biliary cancer fails to reach a weight of the evidence determination and to review the data in the context of their classification scheme. At least IARC noted that the human data for liver and biliary cancer are limited. Failure to highlight how limited the data are in the NTP evaluation leads to the over-interpretation of the data.

For one thing, findings on liver and biliary cancer among TCE exposed workers are not substantial and are in the range of risks that might be explained by one or more forms of unknown bias or confounding. Though elevated across three of the four cohort studies considered to be most useful to IARC, none of the liver and biliary findings are distinguishable from chance (see Table 1). Only one sub-classified finding in these studies is distinguishable from chance and that finding is limited to liver cancers for the workers in the study by Anttila et

Table 1: Risks of Liver and Biliary Cancer Reported in Major Cohort Studies (Significant Increases Denoted with Boldface)

Measure of Risk	Anttila et al. (1995)[a]			Axelson et al. (1994)[a]			Air Force Cohort					
							Spirtas et al. (1991)[b]			Blair et al. (1998)[c]		
	Liver/Biliary	Primary Liver	Biliary Passages	Liver/Biliary	Primary Liver	Biliary Passages	Liver/Biliary	Primary Liver	Biliary Passages	Liver/Biliary	Primary Liver	Biliary Passages
Risk: Overall Men Women	NR	2.27 (0.74-5.29) 3.26 (0.89-8.34) 1.02 (0.03-5.70)	1.56 (0.43-4.00) NR NR	NR	NR 1.41 (0.38-3.60) NR	NR	NR 1.96 (0.85-3.86) —	NR — —	NR 2.38 (0.87-5.19) —	1.3 (0.5-3.4) NR NR	1.7 (0.2-16.2) NR NR	NR
U-TCA[d]: <100 µmol/L 100+ µmol/L	NR	1.64 (0.20-5.92) 2.74 (0.33-9.88)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
U-TCA[e]: 0 - 49 µmol/L 50 - 99 µmol/L 100+ µmol/L	NR	NR	NR	NR	1.89 (0.23-6.83) — —	NR	NR	NR	NR	NR	NR	NR
Cumulative exposure[f] (♂): none <5 5 - 25 >25	NR	NR	NR	NR	NR	NR	NR	NR 3.24 (0.37-12.03) —	NR 2.0 (0.22-7.22) **5.0 (1.00-14.61)** 1.06 (0.01-6.18)	0.5 (0.1-2.4)[g] 1.1 (0.3-4.1)[g] 0.9 (0.2-4.3)[g] 0.7 (0.2-3.2)[g]	NR	NR
Cumulative exposure[f] (♀): none <5 5 - 25 >25	NR	NR	NR	NR	NR	NR	NR	NR — — —	NR 4.35 (0.07-27.82) — 1.43 (0.02-7.95)	4.2 (0.7-25.0)[g] 1.6 (0.2-18.2)[g] — 2.3 (0.3-16.7)[g]	NR	NR
Years since 1st measurement: 0 - 9 years 10 - 19 years +20 years	NR	0.56 (0-6.59)[h] 1.74 (0.21-6.29) **6.07 (1.25-17.7)**[i]	3.28 (0.4-11.8) 0.75 (0.02-4.17) 1.62 (0.04-9.02)	NR	NR	NR	NR	NR	NR	NR	NR	NR
By degree of exposure (♂): Low level intermittent Low level continuous Frequent peaks	NR	NR	NR	NR	NR	NR	NR	NR	NR	1.3 (0.4-4.1) 0.6 (0.2-2.6) 0.7 (0.2-3.0)	1.6 (0.2-14.5) — 0.7 (0.1-11.0)	NR
By degree of exposure (♀): Low level intermittent Low level continuous Frequent peaks	NR	NR	NR	NR	NR	NR	NR	NR	NR	2.8 (0.4-20.3) — 1.8 (0.2-13.0)	— — —	NR

[a] Risk presented as SIR (Standardized Incidence Ratio)
[b] Risk presented as SMR (Standardized Mortality Ratio)
[c] Risk presented as RR (Rate Ratio).
[d] Based on personal urinary TCA level; 100 µmol/L = 16.3 mg/L..
[e] Based on personal urinary TCA for men with greater than 2 years exposure and 10 years latency.
[f] Exposure based on sum of time in each job type and level of exposure for that job. CI for Spirtas et al. (1991) are estimated.
[g] Blair et al. (1998) also present SIRs for cumulative exposure to TCE.
[h] No deaths observed; SMR and CI were estimated by authors.
[i] This increase was observed in males only, with SIR=13.0 (2.68-37.9) for men and no deaths observed in women with +20 yrs since first measurement.

al. (1995) with 20 or more years latency, based on only three cases in men. Only a portion of the biliary cancers in the Finnish cohort studied by Anttila et al. (1995) are reported (those associated with gall bladder and biliary ducts, ICD 155.1), so it is not possible to determine the overall findings for liver and biliary cancers.

The data underlying IARC's assessment of limited data are indeed weak, and NTP should characterize their limited nature more thoroughly. A recent update by Blair et al. (1998) of the cohort studied by Spirtas et al. (1991) highlights the peril to sound judgment of relying on data involving such few deaths. Blair et al. (1998) report SMRs only for the full cohort. Spirtas et al. (1991) reported SMRs for both the full cohort and the TCE subcohort. In Blair et al. (1998) the TCE subcohort is analyzed in comparison to an internal cohort by means of regression modeling using a Poisson distribution model.

Spirtas et al. (1991) reported an SMR for biliary cancer for white workers in the full cohort of 155 (172 for biliary cancer and 111 for liver). Blair et al. (1998) report the comparable SMR to be 110 (115 for primary, and although not reported, the SMR for biliary cancer appears to be 109, 20 observed and 18.4 expected). With further study, and more deaths, these risks have declined.

The use of internal controls and modeled risks for liver and biliary cancer among TCE exposed workers in the study by Blair et al. (1998) complicates interpretation of the findings because there are only four liver cancer deaths among the TCE exposed and one in the unexposed workers. Models with few deaths in either the numerator or denominator can give rise to highly unstable estimates of risk. The relative risk for liver cancer was 1.7, and the more stable estimate of risk for liver and biliary cancer was 1.3. Neither are distinguishable from chance. No risk of biliary cancer was reported, although the earlier findings reported by Spirtas et al. (1991) suggested that biliary cancers, not liver, were elevated.

The specific risks for biliary cancer are not reported. Since the biliary cancers were elevated in the earlier study by Spirtas, but the liver cancers were not, the only reliable basis for comparison is the overall figure of 1.3 for liver and biliary cancer between the studies. Spirtas reported an SMR of 196 (equivalent to 2.0 in the same format) for liver and biliary cancer, and the relative risk in this follow-up report is 1.3. The risks have appeared to decline with more deaths in the TCE subcohort as well as the full cohort.

Blair et al. (1998) also analyzed the risk ratios across categories of cumulative exposure and type of exposure (intermittent low level, continuous low level, and frequent peaks). In neither analysis is a dose-response relationship indicated for liver and biliary cancer. The data on dose indicate that the cohort with the highest doses among the cohort studies is not the Finnish cohort studied by Anttila, but the Swedish cohort studied by Axelson et al. (1994). Yet, the findings in Axelson et al. (1994) do not support a strong association with liver and biliary cancer (SIR of 1.4 based upon four cases).

Anttila et al. (1995) divided the biological data on dose at 100 μmol/L of urinary TCA, a metabolite of TCE (and perchloroethylene). This cutoff represents a division for the higher dose of one-third of the observed deaths and corresponds to a concentration of approximately 6.5 ppm

of TCE in air (1.2 mg/M^3). Axelson et al. (1994) stratified on the highest internal dose of 100 mg/L or more. This is 0.72 mmol/L, or 720 µmol/L or more. Only eight percent of deaths were in this category. The mid-range in dose represents a little over 17 percent of the deaths and the exposure cutoff is 0.36 mmol/L or 360 µmol/L. Together these suggest that the doses in the cohort studied by Axelson et al. (1994) were higher than those in the cohort studied by Anttila et al. (1995) by as much as three times. Both the available information from Blair et al. (1998) and the data available on dose suggest the peril in relying upon the limited data on liver and biliary cancers to imply that these cancers are reasonably anticipated in humans from high occupational exposure to TCE.

NTP mentions the paper by Weiss (1996), and even refers to his emphasis on the combined findings for liver and biliary cancer, as well as for other sites, as "quite limited ... both in terms of the small relative increases seen and the small number of observations upon which the observations are based..." (Weiss 1996). The apparent decrease in risk for liver and biliary cancers indicated by Blair et al. (1998) and the lack of support for dose-response only underscores the necessity for assessing the literature as a whole and using caution in interpreting the human literature on liver and biliary cancer.

Animal Data

NTP relies predominantly on IARC's conclusion that there is sufficient evidence that TCE is carcinogenic in experimental animals. NTP specifically cites mouse liver tumors, both benign and malignant, as support for tumor site concordance between animals and humans. Furthermore, NTP states that humans are similar to mice in their ability to metabolize TCE:

> "Although humans appear more similar to mice than to rats in their ability to oxidatively metabolize TCE, they metabolize approximately 60 times less TCE on a body weight basis than mice at similar exposure levels, and TCA does not appear to induce peroxisome proliferation." (NTP 1998, p. 7-1)

We disagree that humans appear to be more like mice. Humans are more similar to rats than mice in how they metabolize TCE. IARC (1995a) states:

> "A quantitative comparison of the metabolism of trichloroethylene in humans and rats and mice by application of physiologically based pharmacokinetic models suggests that humans have a lower rate of metabolism (14.9 mg/kg bw per h) than B6C3F1 mice (23.2 mg/kg bw per h in females and 32.7 mg/kg bw per h in males) but a slightly higher rate than Fisher 344 rats (11 mg/kg bw per h) (Allen & Fisher 1993)." (IARC 1995a, p. 117).

Numerous authors support the idea that there is a large difference in the metabolism of TCE in mice compared to humans and the lack of relevance for mouse liver tumors to human carcinogenicity (Ashby et al. 1994, Bentley et al. 1993, DeAngelo et al. 1989, Goldsworthy and Popp 1987). NTP should include a discussion of these data in their evaluation of mouse liver tumors.

There are several issues that influence the induction of mouse liver tumors that NTP should take into account. First, B6C3F1 mice are highly susceptible to spontaneous and chemical-induced liver tumorogenesis compared with other rodents or humans. The observation of liver tumors in B6C3F1 mice lacks relevance to humans because of the very high background incidence of liver tumors in these mice, especially when compared to the low rates in humans.

Studies have shown that B6C3F1 mice have a deficiency in the ability to maintain normal DNA methylation (Goodman 1998, Counts et al. 1997, Ray et al. 1994). In comparison, human cells are much more efficient in maintaining normal patterns of DNA methylation (Goodman 1998). These findings have called into question the relevance of B6C3F1 mice liver tumor data in assessing human health risks. Goodman (1998) notes the limitations of B6C3F1 mouse tumor data for predicting human cancer risk:

> "It is not appropriate to make human risk assessment decisions based on a mouse liver tumor response. However, in those situations where the results of a bioassay indicate that the mouse liver is one of several sites where an increased tumor incidence occurs, the mouse liver tumor response and other target sites for tumorogenesis should be evaluated with regard to the mode of the action of the chemical in question." (Goodman 1998, pp. 285-288)

Second, the contribution of peroxisome proliferation to the induction of liver tumors in mice was inadequately evaluated by NTP. Considerable data support the lack of relevance of peroxisome proliferation for humans, particularly if TCA is the key metabolite in the induction of liver tumors. At low to moderate concentrations, TCE is converted to TCA in rodents and TCA has been shown to cause peroxisome proliferation in the mouse liver (Goeptar et al. 1995). Although TCE is also converted to TCA in rats, the oxidative pathway for the conversion of TCE to TCA becomes saturated at much lower concentration levels than in mice. Therefore, the concentrations of TCA necessary to induce peroxisome proliferation in the rat liver are not reached and this explains why rats are not susceptible to developing liver tumors following prolonged exposure to TCE. Comparison of the metabolism of TCE to TCA in human and rodent hepatocytes has shown that human hepatocytes produce much lower levels of TCA than either mouse or rat hepatocytes and, unlike rodent hepatocytes, human hepatocytes do not undergo peroxisome proliferation in response to TCA exposure (Elcombe 1985). In addition, the peroxisomes induced in mice are deficient in catalase as compared to other rodent species. This deficiency may allow increased levels of oxidative DNA damage leading to liver tumor formation (Odurn et al. 1988). Indeed, IARC (1995b) concluded:

> "The results of studies of human hepatocytes *in vivo* and *in vitro*, together with the data on effects in experimental animals, suggest that there are marked species differences in response to peroxisome proliferators. Although further studies are desirable, the current literature suggests that compounds that are peroxisome proliferators in rats and mice have little, if any, effect on human liver." (IARC 1995b, p. 11)

Third, other mechanisms of action, perhaps attributable to other metabolites as well as TCA, strongly suggest the species-specificity of liver tumors in mice. Mouse hepatocytes appear to be

more sensitive to the inhibition of gap junction-mediated intercellular communication by TCE than rat hepatocytes (Klaunig et al. 1989). The inhibition of gap junction intracellular communication is thought to inhibit cellular controls that inhibit the growth and development of premalignant cells. Again, these data support the idea that TCE-induced liver tumorogenesis in B6C3F1 mice is a species-specific phenomenon that occurs as a result of biologic differences inherent in the B6C3F1 mouse strain compared to other rodents and humans. Also, there is experimental data that demonstrates that DCA inhibits liver cell apoptosis in B6C3F1 mice (Snyder et al. 1995), which may also partly explain the high increased incidence of liver tumorogenesis in these animals following exposure to TCE. Loss of the normal regulation of apoptosis may result in the survival and growth of genetically damaged or initiated cells. Therefore, the suppression of apoptosis in mouse liver by DCA may promote hepatocarcinogenesis. DCA does not cause liver tumors in F344 rats suggesting that DCA-induced inhibition of liver cell apoptosis in B6C3F1 mice is a species-specific mechanism that may partly contribute to the induction of liver tumors in these mice.

Lastly, carcinogenicity testing at or near the MTD may promote liver carcinogenesis via increased cell death and cell proliferation which is a mechanism that is not relevant for humans exposed to much lower doses of TCE. Carcinogenesis testing of TCE at or near the MTD results in the production of high liver concentrations of TCA and DCA. Both DCA and TCA have been shown to induce hepatocyte cytotoxicity at high concentrations by causing lipid peroxidation. In turn, lipid peroxidation causes the production of DNA-damaging free radicals, which are thought to initiate tumorogenesis. When given equal doses of TCE, B6C3F1 and Swiss mice produce much higher concentrations of TCA and DCA than NMRI mice or rats. This may explain why B6C3F1 and Swiss mice develop liver tumors while rats and NMRI mice do not develop liver tumors in response to TCE exposure. The fact that TCE can induce chromosomal damage in mice, but not in rats or humans parallels these findings. In addition to peroxisome proliferation, TCA and DCA also induce cytotoxicity leading to increased DNA synthesis and cell proliferation (Goeptar et al. 1995). This mechanism is also thought to cause or contribute to the overall increase in the liver cancer incidence observed in Swiss/B6C3F1 mice exposed to high concentrations of TCE. Because rats and humans produce low levels of TCA and DCA, they would be at less risk of developing liver tumors by this mechanism.

Summary

Based on our review of the human and animal data, there is only weak support for a possible association between exposure to TCE and liver cancer. Only one occupational study has demonstrated an elevated and statistically significant risk of primary liver cancer in workers with 20+ years exposure to TCE (Anttila et al. 1995), which is limited by confounding exposures and small number of cases observed. None of the other cohort studies have detected an increased incidence of liver cancer that could be considered distinguishable from chance. Support for the biological plausibility of TCE-induced liver cancer is limited to findings of increased incidence of liver tumors in two strains of mice. Rats, hamsters, and a third strain of mouse do not develop liver tumors following exposure to TCE. Biochemical data suggests that these mouse strains are more sensitive and prone to developing liver tumors. These qualifications should be taken into consideration as support for a species-specific effect for TCE-induced liver tumorogenesis. The fact that liver tumorogenesis was detected in only one species of animal in conjunction with the

limited findings for liver cancer in humans occupationally exposed to TCE indicates that there is little support to base a casual association between exposure to TCE and liver cancer.

B. NON-HODGKIN'S LYMPHOMA (NHL)

Non-Hodgkin's lymphoma (NHL) is the second cancer that NTP cites as support for the carcinogenicity of TCE based on epidemiological studies in workers exposed to TCE. The risks for NHL are described as being moderately elevated in three cohort studies (Spirtas et al. 1991, Axelson et al. 1994, and Anttila et al. 1995) based on the IARC (1995a) review. NTP also reports that IARC noted "a marginally increased risk for NHL and TCE-contaminated groundwater" (Cohn et al. 1994, Vartianen et al. 1993).

Based on our review of these studies, we do not believe the data on NHL justify the characterization presented in the NTP review. The three occupational cohort studies report elevations of NHL, but none of these findings is statistically significant. In other words, these findings could be attributable to chance. In addition, there are inconsistences in the dose-response data, which do not support an association between TCE exposure and NHL. The drinking water studies especially, are limited by design, small numbers, and the absence of a dose-response. Given the limitations of the drinking water studies, their relevance to an overall assessment of the potential association between TCE exposure and NHL is questionable. Finally, only one animal study has reported an increased incidence of lymphomas following TCE exposure (Henschler et al. 1980). We believe that there are deficiencies in the findings on TCE and NHL beyond bias, chance, and confounding that limit the interpretation of an association. We propose that these limitations be described more thoroughly in the overall evaluation of TCE.

Occupational Cohorts

None of the three major epidemiological occupational studies cited by IARC and NTP report a statistically significant increased risk for NHL from TCE exposure (Table 2). By definition, these non-significant findings have not excluded chance as a cause of the elevations. As noted above, Weiss (1996) concluded that "the evidence currently available in support of a causal hypothesis is quite limited" and he recommended that there be additional followup and the study of new cohorts. The Air Force cohort studied by Spirtas et al. (1991) was recently updated by Blair et al. (1998) and also fails to demonstrate a statistically significant association between TCE exposure and NHL.

Given the lack of strong association between TCE and NHL among the occupational cohorts studied, additional data to support a causal association is necessary before TCE could be said to be reasonably anticipated to cause NHL in humans. On the contrary, there are two factors that argue against an association -- the lack of a consistent dose-response and the lack of specificity for TCE exposure and NHL.

First, the data on dose-response in the subcohort analyses of the three major occupational cohorts does not support an association. The subcohort analyses for cumulative TCE exposure in the Spirtas et al. (1991)/Blair et al. (1998) studies do not support a dose-response trend as the men with highest exposure have the lowest risk, 0.57 and 1.1, respectively. Women also had lower

Table 2: Risks for NHL Reported in Major Cohort Studies

Measure of Risk	Anttila et al. (1995)[a]	Axelson et al. (1994)[a]	Air Force Cohort	
			Spirtas et al. (1991)[b]	Blair et al. (1998)[b]
Risk for NHL:				
Overall	1.81 (0.78 - 3.56)	1.56 (0.51 - 3.64)		2.0 (0.9 - 4.6)
Men			1.03 (0.49 - 1.89)	
Women			2.86 (0.78 - 7.31)	
U-TCA[c]:				
<100 µmol/L	2.01 (0.65 - 4.69)	NR	NR	NR
100+ µmol/L	1.40 (0.17 - 5.04)			
U-TCA[d]:				
0 - 49 µmol/L	NR	1.64 (0.20 - 5.92)	NR	NR
50 - 99 µmol/L		1.16 (0.0 - 12.72)		
100+ µmol/L		8.33 (0.22 - 46.43)		
Cumulative exposure[e] (♂):				
none	NR	NR		1.6 (0.5 - 4.5)
<5			1.28 (0.41-2.99)	1.8 (0.6 - 5.4)
5 - 25			1.29 (0.26-3.81)	1.9 (0.6 - 6.3)
>25			0.57 (0.06-2.06)	1.1 (0.3 - 3.8)
Cumulative exposure[e] (♀):				
none	NR	NR		2.0 (0.3 - 12.2)
<5			3.28 (0.04-18.55)	3.8 (0.8 - 18.9)
5 - 25			—	—
>25			3.30 (0.67-9.74)	3.6 (0.8 - 16.2)
Years since 1st measurement:				
0 - 9 years	0.83 (0.02 - 4.64)	NR	NR	NR
10 - 19 years	1.75 (0.48 - 4.47)			
+20 years	3.24 (0.67 - 9.45)			
By degree of exposure (♂):				
Low level intermittent	NR	NR	NR	1.5 (0.5 - 4.3)
Low level continuous				1.8 (0.6 - 5.2)
Frequent peaks				1.5 (0.5 - 4.4)
By degree of exposure (♀):				
Low level intermittent	NR	NR	NR	3.9 (0.8 - 17.7)
Low level continuous				3.4 (0.5 - 21.7)
Frequent peaks				3.8 (0.9 - 16.2)

[a] Risk presented as SIR (Standardized Incidence Ratio)
[b] Risk presented as SMR (Standardized Mortality Ratio)
[c] Based on personal urinary TCA level; 100 µmol/L = 16.3 mg/L.
[d] Based on personal urinary TCA for men with greater than 2 years exposure and 10 years latency.
[e] Exposure based on sum of time in each job type and level of exposure for that job. [Note: estimated C.I.s for Spirtas et al. (1991)]

SMRs in the highest category, with no deaths in the middle category. Blair et al. (1998) also conducted an analysis based on degree of exposure, which failed to show a trend for low level intermittent or continuous exposure, or for peak exposure. Axelson et al. (1994) conducted an analysis of exposure based on urinary TCA concentrations, and all cases, except one, were in the lowest exposure category. The single case in the highest exposure category is insufficient to support causation as it may be due to chance. Anttila et al. (1995) also conducted an analysis by personal urinary TCA levels, which does not support a dose-response trend with an SIR in the low exposure group of 2.0 (C.I. 0.65 - 4.69) and an SIR of 1.4 (C.I. 0.17 - 5.04) in the high exposure group.

Second, both Spirtas et al. (1991) and Blair et al. (1998) evaluated the relative risk for NHL and a wide range of solvents. While the risks for TCE and NHL were elevated, they were no greater than those observed for some of the other solvents. The relative risks for men exposed to TCE ranged between 1.1 and 1.9, with risks for exposure to other solvents ranging between 1.0 and 3.0 (methylene chloride). For women, the relative risks for TCE were between 3.4 and 3.9 and the risks for other solvents ranged between 1.3 and 6.5 (solder flux). As the authors discuss, these analyses are limited by small numbers of deaths and the overlap of exposures. However, it does demonstrate that the elevations of NHL in these workers may not be attributable to their exposure to TCE.

Neither IARC nor NTP discuss Garabrant et al. (1988) or Henschler et al. (1995), other occupational cohorts, which did not identify an increased risk for NHL. Garabrant et al. (1988) did not observe an increased risk for lymphosarcoma and reticulosarcoma, which includes NHL (SMR = 82, 95% C.I. 44 - 141). These 14,067 aircraft workers were exposed to a range of compounds, from which it was estimated that 37 percent of the workers were exposed to TCE. Henschler et al. (1995) only observed one cancer of the lymphatic and hematopoeitic system, a multiple myeloma.

Overall, the weight of evidence for an association between TCE exposure and NHL is extemely limited. The limitations include a weak association in a few studies, but not all studies, and even those associations could be attributed to chance; the lack of a consistent dose-response in the cohorts that evaluated exposure; and possible confounding exposure to other solvents. These limitations severely limit the interpretation of these data.

Drinking water studies

The limitations in the drinking water studies cited by NTP include the ecological design of the studies, the small number of cases, and the absence of a dose-response. These studies do not demonstrate an increased risk for NHL from TCE exposure. If the risks observed in the drinking water studies were real, we would expect to observe even greater risks in the occupational studies where exposures are even higher. The total dose received by workers in the occupational studies are at least 100-fold higher than the TCE dose received if 2 liters of water were ingested per day at the concentrations described in the drinking water studies. Despite doses of TCE that are two orders of magnitude higher than those in the drinking water studies, the association between TCE and NHL remains very weak and inconclusive.

At best, Cohn et al. (1994) report only a suggestive link between TCE/PCE exposure and NHL, and even these findings are limited by the potential for exposure mis-classification and the ecological study design. Groundwater modeling and concentration estimates were only prepared at a town level. Therefore, individual exposures and the potential association with NHL are unknown. The pattern of relative risks reported in Cohn et al. (1994) are not consistent and few are statistically significant. Low-grade and total NHL are statistically significant in men in towns with exposure to 0.1-0.5 ppb TCE, but not in towns with exposure to TCE greater than 0.5 ppb. Several categories of NHL were statistically significant in females, but the highest relative risk observed in high-grade non-Burkitt's lymphoma is based on only six cases. The effect of multiple comparisons in this study is not assessed.

Both IARC and NTP characterize the NHL finding in Vartianinen et al. 1993 to be marginal in one village. However, the authors of the study conclude that "no increased incidence rates were found." In addition, the slightly increased incidence of NHL in Hattula is in contrast to the lower than expected rate for NHL in Hausjärvi/Oitti where the exposure concentrations were higher. This observation does not support a typical dose-response pattern, and further emphasizes the limited nature of the support for a causal association. The drinking water studies constitute even more limited support than the occupational cohort studies.

Animal Study

NTP cites a single experimental animal study in which an increased incidence of lymphomas in female mice was observed following exposure to TCE (Henschler et al. 1980). However, no attempt is made to propose a mechanism of action or to explain why this increase was observed in female mice only.

Henschler et al. (1980) report no increased incidence for any tumors in rats, mice, or hamsters except for the finding of lymphomas in female mice. This type of lymphoma is known to occur spontaneously with a high rate of incidence, particularly in female mice. In fact, the authors acknowledge that there is a high spontaneous incidence of lymphomas in female mice and conclude that the:

> "[I]ncrease in the lymphoma rate in female mice resulting from almost lifelong inhalation of a high concentration of trichloroethylene cannot be taken as proof of a carcinogenic potential." (Henschler et al. 1980, pp. 245 -246)

NTP does not discuss the other cancer bioassays on TCE (NTP 1988, 1990). NTP (1990) report an increase in lymphomas, but it was concluded that:

> "The increased incidence of dosed female mice with malignant lymphoma ($p<0.5$) and lymphoma or leukemia are not considered to be related to the administration of TCE." (NTP 1990, p. 56)

Given the lack of support for a link between the TCE exposure and the incidence of lymphomas in Henschler et al. (1980) and NTP (1990) and the absence of data on lymphomas in other experimental studies, the data are easily insufficient to support a causal association between TCE

exposure and lymphomas in experimental animals. The animal data for TCE and NHL cannot be considered to be relevant to humans because of the high background rate for lymphomas in female mice.

Summary

Small elevations in NHL have been observed in workers exposed to TCE and in ecological studies of populations exposed to TCE in the drinking water. However, the limitations in these studies restrict their applicability in assessing a causal relationship between TCE exposure and NHL. The weak, non-significant association in some studies, the lack of a consistent dose-response, and the potential for confounding exposures all suggest that these observations may have occurred by chance. In addition, there is no animal data to support this association, nor are there any data on a possible mechanism that is relevant to humans. We propose that these limitations be highlighted and fully described in the NTP summary of their assessment of TCE.

C. KIDNEY CANCER

NTP implies that TCE is associated with renal cancer in humans (p. RC-1), citing a retrospective cohort study by Henschler et al. (1995), a human molecular study by Brüning et al. (1997), and three animal studies (Maltoni et al. 1988, NTP 1988, NTP 1990), which found increased incidence of renal tumors in rats exposed to high levels of TCE. These studies only provide a preliminary indication that TCE may cause kidney cancer in humans. These data in conjunction with the other TCE cohort studies suggest that if TCE does induce kidney tumors it is a threshold, high-dose phenomenon. When discussing the data in favor of the association of occupational exposure to high concentrations of TCE and kidney cancer, NTP should provide a balanced presentation of these data by noting their limitations and the fact that other cohort studies do not report an association between kidney cancer and TCE exposure.

Human data

NTP cites only one epidemiological study, Henschler et al. (1995), as support of a possible association between kidney cancer and occupational exposure to TCE. Henschler et al. (1995) is a relatively small cohort of TCE exposed workers (n=169) compared with the other TCE cohort studies that have been conducted. NTP also cites Brüning et al. (1997), a human molecular study, as possible support for the association between kidney cancer and TCE exposure. Some of the patients evaluated in the Brüning study are likely to have been a part of the Henschler et al. (1995) cohort, and therefore, this does not constitute an independent report of kidney cancer in humans occupationally exposed to TCE.

Several epidemiological studies involving large cohorts of workers exposed to TCE have evaluated kidney cancer and have not observed an elevation in kidney cancer incidence or mortality. Blair et al. (1998), the recent update to Spirtas et al. (1991), did not report an elevation of kidney cancer in men and women with occupational exposure to TCE (RR= 1.6, 95% C.I. 0.5 - 5.1) based on regression modeling using an internal comparison group. Axelson et al. (1994) did not find an increased incidence of kidney cancer in a cohort study of men occupationally exposed to TCE (SIR= 116, 95% C.I. 42 - 252). Anttila et al. (1995), who verified exposure to

TCE by urine TCA measurement, did not observe an increase in the incidence of kidney cancer in men and women with occupational exposure to TCE (SIR=87, 95% C.I. 32 - 189).

IARC (1995) focused their assessment on the above three occupational cohorts and included the Henschler et al. (1995) study in their review, but did not give it equal weight in its assessment of the carcinogenicity of TCE. In part, the reason for the not giving Henschler et al. (1995) equal weight is due to the criticisms voiced about the methods used and the fact that the incidence data appear to be derived from a cancer cluster (Swaen 1995, Bloemen and Tomenson 1995, McLaughlin and Blot 1997, Weiss 1996). Three of the original five kidney cancer cases should have been excluded from the Henschler incidence analysis based on the accepted guidelines for analyzing hypotheses generated from cancer cluster findings (McLaughlin and Blot 1997). If three of the cases are excluded from the analysis, the incidence of renal cancer in this population is not statistically significant. Not all the renal tumors observed in the Henschler cohort were of the same histological type (pelvic versus renal cell) which calls into question the proposed association between the various tumors and exposure to TCE. Kidney cancer deaths among the Henschler cohort were similar to expected (SMR=0.63 for exposed; SMR=0.61 for expected) Although the cure rate for kidney cancer is quite high today and might explain a deficit between kidney cancer incidence and deaths in this cohort, at the time period covered by the study (1956-75) the cure rate for this disease was only 45-50 percent. As a result, the low death rate for kidney cancer among the TCE-exposed workers in the Henschler cohort cannot be explained by a high cure rate (McLaughlin and Blot 1997).

Brüning et al. (1994) is simply a study of potential molecular markers for TCE-induced kidney cancer in humans and in no way provides definitive epidemiological data for an association between exposure to TCE and kidney cancer. Brüning et al. (1994) found that all 23 patients with exposure to TCE had one or more of three kinds of somatic mutations located in the *VHL* gene. As noted by the authors, the incidence of this mutation in these patients is high compared to previous studies in other renal cancer patients. However, the authors did not use a control population for this study. Therefore, it is not possible to conclude that the *VHL* mutations are a result of TCE exposure. Somatic mutations in the *VHL* gene are common in patients with *a hereditary form of renal cancer* (Linehan et al. 1997), but *VHL* mutations have not previously been associated with exposure to environmental carcinogens. Furthermore, it is not clear if mutations in the *VHL* gene are the cause in renal cancer or are a middle- to late-stage molecular change in the maturing tumor. NTP should note the limitations of this study along with the alternative causes of the observed mutations in the *VHL* gene.

Animal data

The animal studies conducted by Maltoni et al. (1988) have several problems that are not raised in the NTP Background Document. Treated and untreated animals were held until spontaneous (natural) death, which is not a procedure advocated by the NTP for carcinogenesis testing. Secondly, pathological examinations were not verified by independent observers and the pathological results have been criticized on the basis that appropriate pathological data were not reported when tumors occurred (ATSDR 1997). It is also not apparent from the study results if Good Laboratory Practices were followed. IARC (1995a) notes that the difference in kidney tumor response in males versus females in the Maltoni study "...begs caution in the interpretation

of the results." The NTP (1988) TCE cancer bioassay suffers from poor survival in all dose groups both from exposure to TCE and what IARC (1995a) calls "accidental deaths." Therefore, this study is of questionable value for evaluating the ability of TCE to induce renal cancer in rodents. The NTP (1990) study also suffered from significantly reduced survival among both treated and control animals. There was a small but statistically significant increase in the incidence of renal tubular cell adenocarcinomas in male rats but no treatment-related increase in the incidence of tumors was observed in female rats. The findings of this study were judged to be equivocal by the NTP investigators (NTP 1990).

A number of animal studies have failed to show an increase in the incidence of renal tumors following chronic administration of TCE. The incidence of renal tumorogenesis was not increased in B6C3F1 mice treated with TCE orally (NCI 1976), although it is noted that there was low survival in both controls and in all dosage levels. Exposure of NMRI mice or Syrian hamsters to TCE in air did not increase the incidence of renal tumors in these animals as compared to controls (Henschler et al. 1980) and Sprague-Dawley rats exposed to TCE in air did not develop an increased incidence of renal tumors as compared with controls (Fukuda et al. 1983). However, we note that survival in the control groups for Fukuda et al. (1983) was lower than in the exposed groups at 100 weeks (50 versus 75 percent).

Mechanistic data

In NTP's discussion of TCE metabolism, no mention is made of the fact that the pathway involving conjugation of TCE with glutathione is a high-dose phenomenon that is not expected to occur unless the oxidative pathway is saturated (Goeptar et al. 1995). At high doses, TCE is conjugated with glutathione. This alternate pathway occurs only with the saturation of the major, cytochrome P-450 pathway. The glutathione conjugate is then converted to dichlorovinylcysteine (DCVC) by β-lyase in the kidney (Goeptar et al. 1995). DCVC is thought to be the metabolite that causes renal toxicity and carcinogenicity in male rats following exposure to high doses of TCE (Goeptar et al. 1995).

The observation of recurrent injury with kidney tumors in rats at very high doses reduces the concern for possible generation of DCVC metabolites in humans at typical and lower doses. DCVC metabolites are nephrotoxic at higher doses (Lash et al. 1986, Bruckner et al. 1989, Dekant et al. 1989, Green et al. 1990, IARC 1995a, Goeptar et al. 1995). Chronic nephrotoxicity is present in both Fischer 344 and Osborne-Mendel rats to a substantial degree (70-90% of animals) at doses, such as 1,000 mg/kg. Such high doses lead only to a tumor incidence of approximately 2-6 percent. This substantial level of recurrent cytotoxicity would be capable of fixing and promoting any genotoxic damage that is induced by reactive thiol intermediates formed upon degradation of DCVC by beta-lyase. Thus, chronic and severe nephrotoxicity is believed to be a prerequisite, but not a particularly effective one, for the nephrocarcinogenic effects of TCE in the rat (Dekant et al. 1989, Goeptar et al. 1995, Brüning et al. 1996). The importance of such chronic injury is underscored because renal cancer has not been observed in the rat in the absence of severe and chronic kidney injury (Dekant et al. 1989, Goeptar et al. 1995).

By comparison, mice produce DCVC metabolites, but do not develop kidney tumors. This strongly suggests that the genotoxicity of the DCVC intermediates, which are subsequently bioactivated by beta-lyase, is insufficient by itself to produce renal tumors in other rodent species. Thus, the kidney tumors are induced in rats from a combinations of events unique to the rat, and that recurrent and severe nephrotoxicity is a key element (Goeptar et al. 1995, Yoshikawa 1996). For humans, the chronic nephrotoxicity necessary for production of tumors in rats would not exist at lower doses of the DCVC metabolites that are likely at the doses typical of human exposures to TCE. Thus, it is mechanistically improbable that TCE is capable of inducing kidney cancer in humans at low doses (Dekant et al. 1989, Selden et al. 1993, Goeptar et al. 1995, Brüning et al. 1996).

Differences in the metabolism of TCE to DCVC explains the difference in tumor response between male and female rats and the insensitivity of mice to the renal carcinogenicity of TCE, given the much higher oxidative capacity of mice. GSH and β-lyase activity in the male rat is much higher than the female rat or in mice of either sex (Goeptar et al. 1995). Compared with rodents, humans appear to have a lower capacity to initiate the GSH pathway and consequently are at lower risk for renal carcinogenesis (Green et al. 1990, Goeptar et al. 1995). Several other differences in the activity and enzymes necessary for this pathway suggest that humans are less likely to metabolize TCE via the GSH pathway (DeKant et al. 1989, Goeptar et al. 1995, Green et al. 1990, Hinchman and Ballatori 1990). Thus, the lower metabolic activity for humans supports only a potential for kidney carcinogenesis in humans at very high exposures.

Summary

Undue emphasis is placed on the possible association between TCE exposure and kidney cancer in the NTP Background Document. Only one cohort study provides possible support for an association between kidney cancer and humans. Many criticisms of this study are in the literature, and therefore, it is of questionable value until the data can be confirmed by other studies. Several large cohort studies conducted to date have not found an association between occupational exposure to TCE and kidney cancer. Thus, using a weight of evidence approach, there is insufficient data to link TCE exposure and kidney cancer. The three animal studies that have reported an increase in the incidence of kidney cancer, observed this finding in male rats only. In addition, there are other limitations regarding the methods used and the survival rates in the animal studies. Finally, metabolic data suggests that if exposure to TCE is associated with the development of kidney cancer, it is anticipated to be a threshold, high-dose phenomenon that is not expected to occur in populations with exposure to low to moderate concentrations of TCE. Therefore, we propose that NTP acknowledge these data limitations in the executive summary by including their conclusion regarding the Henschler et al. (1995) study that:

> "In summary, the concerns raised about the Henschler et al. (1995) study have generally limited its usefulness for assessing the causal evidence for the human carcinogenicity of TCE..." (NTP 1998, p. 3-4).

D. OTHER TUMOR SITES

NTP references several other cancer sites as being possibly associated with TCE exposure. The two sites that we will comment on are the lung tumors in mice and the epidemiological data for melanoma.

Lung tumors

NTP states that the epidemiological data was predated by studies in experimental animals (RC-1). They follow this statement with a list of several tumors reported in the animal studies, including mouse lung tumors. None of the occupational cohorts report an increased risk for lung cancer. The presentation in the summary suggests that there is site concordance for lung tumors. We propose that this discussion be clarified to be more specific about which tumors are considered to be concordant and those which are not. In addition, NTP should discuss the animal studies which did not report an increased incidence of lung tumors.

NTP briefly discusses the limitations of the data on TCE exposure and lung tumors in mice, but these are not carried forward into the overall summary of findings. The issues that we believe are relevant in the interpretation of these data are the fact that this increased incidence is observed in only two strains of mice, an increase was not observed in three other studies, there are gender differences in the incidence of lung tumors, and the metabolic data provide support that these tumors are not relevant to humans.

The NTP summary states that increased incidence of lung tumors was observed in two strains of mice in three studies. However, NTP does not highlight the importance of the strain and specie-specific character of these observations. Two other studies in mice were not discussed that failed to report a significant increase in the incidence of lung tumors (Henschler et al. 1980, NTP 1990). NTP (1990) reported a significantly increased incidence of alveolar/bronchiolar adenomas in female mice, but they were no longer significant when evaluated combined with carcinomas. Similar studies conducted on rats and hamsters did not report an increased incidence of lung tumors. When Syrian hamsters were exposed to TCE, histopathological examinations of the lung revealed no significant increase in tumor incidence. A similar study conducted on groups of Wistar rats also revealed no increase in lung tumor incidence (Henschler et al. 1980).

The increased tumor incidence was observed in *female* B6C3F1 mice and *male* Swiss mice. This denotes an additional gender- and strain-specific mechanism of action at work. While NTP acknowledges that the findings of lung tumor incidence in mice are gender- and specie-specific, it does not specifically state that the metabolic process responsible for pulmonary damage does not occur in the human lung. Green et al. (1997) elaborate:

> "[T]he metabolic processes leading to the cellular damage have been shown, by three techniques, not to occur in human lung...there is now strong evidence to suggest that lung tumors seen after exposure to TRI [TCE] are a mouse-specific phenomenon that does not occur in rats and will not occur in humans." (Green et al. 1997, p. 129)

Thus, if the lung tumors are strain, specie- and gender-specific and not relevant to human carcinogenicity, there is no basis for relying on lung tumors for the carcinogenic classification of TCE. Specifically, there is no justification for inclusion of the lung tumor data as support for TCE carcinogenicity in humans in the Executive Summary. If the lung tumors are included in the overall assessment, they should be included only as part of the summary of data on TCE and an understanding of the mechanism of action for these tumors should be fully described.

Melanoma

In their summary of the human epidemiology data, NTP includes a summary of a recent case-control study by Fritschi and Siemiatycki (1996) that investigated the occurrence of malignant melanoma in relation to potential exposure to 85 different substances, 13 occupations, and 20 industries. This study found increased risk of malignant melanoma in association with exposure to TCE based on eight exposure cases. No other studies are cited by NTP that have found an association between exposure to TCE and increased risk of malignant melanoma. Appropriately, these data were not included in the Executive Summary.

We would like to emphasize that Fritschi and Siemiatycki(1996) has limited statistical power to detect a true association based on the limited number of cases (8 with "any" exposure to TCE; 4 with "substantial" exposure to TCE). In fact, there is no clear dose-response in their assessment of "insubstantial" exposure to TCE (RR = 3.8, 95% C.I.: 1.1-13.6) and "substantial" exposure to TCE (RR = 3.4, 95% C.I.: 1.0-12.3).

A previous study by Siemiatycki(1991), using the same cases and controls as Fritschi and Siemiatycki(1996), found a lower risk of malignant melanoma among four cases with "substantial" exposure to TCE (RR = 2.3, 95% CI: 0.9 - 5.8) or eight cases with "any" exposure to TCE (RR = 2.6, 95% C.I.: 1.3 - 5.0). The analyses performed by Fritschi and Siemiatycki(1996) differ from Siemiatycki(1991) in that the number of "controls diagnosed with cancer" was reduced from 2,525 to 533 to provide equal weight between population controls (n=533) and cancer controls (n=533). However, the authors do not provide details on the methods used to reduce the cancer control group. The unexplained reduction of the cancer control group is a possible source of selection bias which could potentially elevate the perceived risk between malignant melanoma and occupational exposure to TCE.

Other studies of larger populations exposed to TCE have not reported significantly elevated risk of malignant melanoma. Anttila et al. (1995) did not evaluate melanoma in the TCE-exposed workers and did not observe an increased risk for melanoma in the full cohort of workers exposed to halogenated hydrocarbons (SIR = 0.71, 95% C.I. 23-166). Spirtas et al. (1991) did not find an increase in the risk of death for malignant melanoma for men (SMR = 96, 95% C.I. 31 - 224) or women (1 obs., 0.6 exp., SMR not calculated). Blair et al. (1998), a recent update of Spirtas et al. (1991), also did not observe an increased risk for melanoma (RR = 1.0, 95% C.I. 0.3 - 3.1). Garabrant et al. (1988) did not report an increased risk for malignant melanoma (SMR = 71, 95% C.I. 29 - 148). Axelson et al. (1994) report a marginally significant increase in skin cancer, but non-melanotic (SIR = 3.4, 95% C.I. 1.02 - 4.65), not melanoma.

The weight of evidence for melanoma and TCE exposure is of no association. Only one case control study, with potential selection bias, observed an increased risk for melanoma. None of the large occupational cohort studies observed an increased incidence of melanoma. In addition, none of these studies take into account the most important confounding factor for melanoma, average exposure time to sunlight.

IV. MISCELLANEOUS COMMENTS

NTP states that the findings of Lipscomb et al. (1997) demonstrate a "lack of uniformity" among the human samples and that sensitive sub-populations may exist with increased susceptibility to TCE. The "lack of uniformity" observed in the Lipscomb et al. (1997) was less than an order of magnitude and is what is expected given the normal variability among humans. This study, in fact, confirms and supports the typical application of a 10-fold safety factor in risk assessments of non-cancer endpoints to account for variability within the human population.

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